

Shell-Vial Culture and pp65 Antigenemia Assay in the Detection of Cytomegalovirus in the First Blood Sample of Renal Transplant Recipients

Jordi Reina,* Victoria Fernandez-Baca, Juan Saurina, Isabel Blanco, and Maria Munar

Virology Unit, Clinical Microbiology Service, University Hospital Son Dureta, Universitat Illes Balears, Palma de Mallorca, Spain

The aim of the study was to compare the efficacy of pp65 antigenemia assay and the shell-vial culture (SVC; viremia) for the diagnosis of cytomegalovirus (CMV) infection in renal transplant recipients, comparing the results obtained in the first blood sample and the total number of blood samples analyzed in this group of patients. During the study period, 70 renal transplant recipients were studied: 44 (62.8%) with CMV infection. The method of sedimentation in a dextran solution for leukocyte extraction was used in the pp65 antigenemia assay. The MRC-5 shell-vial assay was used for CMV isolation from leukocytes (viremia).

Eighty blood samples were examined from 70 renal transplant recipients: Of the 44 positive samples studied, in 77.5% of cases, both the antigenemia assay and the SVC were positive. In 16.2%, only the antigenemia assay was positive, and, in 6.2%, only the SVC was positive. In all blood samples studied, the antigenemia was present in 93.7% of cases, and the SVC was present in 83.7% ($P = 0.04$). If the results obtained in only the first blood sample taken for the diagnosis are studied, then we observe that the antigenemia assay was positive in 39 patients (88.6%), whereas the SVC was positive in 41 patients (93.1%), although the difference was not statistically significant ($P = 0.39$). It is concluded that the inoculation of all of the leukocytes extracted from blood samples in the SVC seems to produce a slight increase in the sensitivity of the cell culture and that the SVC becomes positive before the antigenemia for the detection of CMV in peripheral blood, especially in the first blood sample. *J. Med. Virol.* 55:240–242, 1998.

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INTRODUCTION

Infection by cytomegalovirus (CMV) is one of the main causes of morbidity and mortality in immunosuppressed patients. Because there are drugs available with proven efficacy against CMV, an early diagnosis of infection is very important in order to initiate specific therapy [Rubin, 1988, 1990; Martin, 1995].

Of the various techniques used for the diagnosis of infection by CMV, the detection of the pp65 antigen for this virus in the leukocytes of peripheral blood (antigenemia) and culture of CMV in blood (viremia) are the methods that have displayed the best correlation with the presence or the immediate development of infection or disease by CMV [Van den Berg et al., 1989; Wunderli et al., 1991; Landrey and Ferguson, 1993].

Most comparative studies seem to show that antigenemia is easier to detect than the shell-vial culture (SVC) for rapid detection of CMV in leukocytes [Van der Bij et al., 1988; Buller et al., 1992; Erice et al., 1992]. However, in these studies, the same volume that was used for the antigenemia or a fixed volume is inoculated into the SVC [Van der Bij et al., 1988; Erice et al., 1992; Landry and Ferguson, 1993]. A prospective, comparative study of the efficacy of pp65 antigenemia assay and SVC was undertaken for diagnosis of CMV infection in renal transplant recipients.

MATERIALS AND METHODS

During the study period, 70 renal transplant recipients were studied, of whom 44 (62.8%) were with suspected CMV infection (fever, leukopenia). Antigenemia assay and SVC were carried out on the first blood sample taken from each patient between the 20 and 45 days posttransplant, and the results were compared. Once the results from the first blood sample were

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*Correspondence to: Jordi Reina, Virology Unit, Clinical Microbiology Service, University Hospital Son Dureta, Universitat Illes Balears, 07014 Palma de Mallorca, Spain.

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known, patients were followed up with other antigenemia assays and blood cultures.

Sedimentation in a dextran solution for leukocyte extraction was used following the recommendations of Gerna et al. [1992], with minor modifications. Briefly, 3 ml of heparinized whole blood were mixed with 6% dextran solution in saline (Macrodex, Pharmacia, Sweden). Following incubation at 37°C for about 30–45 minutes, 1.0 ml of supernatant rich in leukocytes was collected, mixed with 3.0 ml of phosphate-buffered saline (PBS), and centrifuged at $\times 200g$ for 10 minutes at room temperature. The supernatant was discarded, and the pellet was resuspended in 4.0 ml of PBS. The sample was divided in two parts of 2.0 ml each. The portion intended for the SVC was mixed with 2.0 ml of PBS and centrifuged. The supernatant was discarded, the pellet was resuspended in 1.0 ml of PBS, and the cells were counted in a hematological counter. The leukocytes were inoculated in two MRC-5 shell-vials (500 μ l per vial; Vircell, Ingelheim Diagnostica, Spain). The MRC-5 shell vials were centrifuged at $\times 700g$ for 45 minutes, incubated at 37°C for 18–24 hours, and then stained by an indirect immunofluorescence assay with a monoclonal antibody against the p72 CMV antigen (clone E13; Argene Biosoft, France).

The antigenemia assay was carried out as described previously [Reina et al., 1996]. Briefly, cytopins of leukocyte preparation were obtained by centrifugation of 2×10^5 cells onto glass slides at 700 rpm for 10 minutes by using a cytocentrifuge (Cytospin 3; Shandon Scientific, United Kingdom). The slides were fixed with formalin for 10 minutes, washed in PBS for 5 minutes, and allowed to dry. After fixation, slides were stained by an indirect immunofluorescence assay with a monoclonal antibody against CMV pp65 (Monofluokit CMV; Diagnostics Pasteur, France). The slides were read and counted under fluorescence microscopy at a magnification of $\times 40$.

Statistical analysis was carried out on results of different comparisons by using Student's *t*-test on paired data. All *P* values were two-tailed and were considered significant if they were less than 0.05.

RESULTS

During the study period, 80 blood samples from 70 renal transplant recipients were examined. Of the 44 positive samples studied, it was observed that, in 62 patients (77.5%), both the antigenemia and the SVC were positive; in 13 patients (16.2%), only the antigenemia was positive; and, in 5 patients (6.2%), only the SVC was positive. In all blood samples studied, the antigenemia was positive in 93.7% of cases, and the SVC was positive in 83.7% of cases, with a statistically significant difference (*P* = 0.04).

From the results obtained in the first blood sample taken for the diagnosis of infection by CMV were examined, it was observed that the antigenemia was present in 39 patients (88.6%), whereas the SVC was positive in 41 patients (93.1%). The difference was not statistically significant (*P* = 0.39). In three patients

(6.8%), the antigenemia was positive, and the SVC was negative; and, in five patients (11.3%), the SVC was positive, and the antigenemia was negative.

The quantities of leukocytes inoculated in the shell vials varied between 150,000 and 1,200,000 per vial. Only a partial toxic effect on the monolayers was observed, and it was not necessary to discard any sample, because none was completely destroyed.

DISCUSSION

The majority of studies comparing the diagnostic efficacy of the antigenemia assay and the SVC or the conventional (tube) culture fail to establish the quantity of leukocytes inoculated in the cell culture. In general, the same quantity of leukocytes as that used for the antigenemia assay is used, or a fixed-volume extraction (0.2–0.3 ml) is added [Van der Bij et al., 1988; Erice et al., 1992; Landry and Ferguson, 1993]. Consequently, the antigenemia assay shows much greater sensitivity than the cell culture in the rapid detection of CMV in peripheral blood [Van der Bij et al., 1988; Erice et al., 1992].

In this study, two diagnostic techniques were compared in two different situations. First, they were compared from the first diagnostic sample and, thus, in the absence of factors that could negate the cell cultures (antiviral treatment). Under these circumstances, it was observed that the SVC was as sensitive as the antigenemia assay for the detection of CMV in peripheral blood (*P* = 0.39). In five patients, the viremia was positive, and the antigenemia assay was negative in the first sample. In all samples, both techniques gave positive results in samples that were taken later, 24 hours after the first sample. In this way, the inoculation of all extracted leukocytes in the SVC determined that the diagnosis of viremia by CMV could be obtained faster than if only quantities of leukocytes equal to those used for the antigenemia assay were cultured.

In general, after the detection of CMV in blood samples, the majority of patients receive specific pre-emptive therapy in order to avoid the development of CMV disease [Van den Berg et al., 1989; The et al., 1992]. These patients are followed up during treatment, and the development and appearance of antiviral resistance is monitored by means of a succession of blood samples, which, despite the fact that some are still positive in the antigenemia assay, are negative in the culture. In this way, all of the samples from these patients are examined, and a greater sensitivity of the antigenemia assay has been found, the difference being significant (*P* = 0.04). In summary, the inoculation of all of the leukocytes extracted from blood samples in the SVC culture makes this assay as sensitive as the pp65 antigenemia.

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